ORIGINAL RESEARCH

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Comparison between arterial and venous sampling of circulating hormones, substrates and peptides in severe obesity

Abstract

Purpose: Severely obese patients are being encountered more frequently in clinical practice. Factors implicated in the relationship between obesity and cardiovascular disease may be measured from a blood sample obtained through arterial access in a cardiology setting, such as during cardiac catheterization or heart surgery. The comparability of a given sample site (arterial *vs.* venous) with regards to blood parameters is yet to be established.

Methods: Fifteen severely obese patients undergoing bariatric surgery were recruited. Fasting blood samples were collected simultaneously from the radial artery (A) and the superior vena cava (V), both representing general circulating levels, after anesthesia but before the surgical procedure. Blood samples were analysed for glucose, insulin, non-esterified fatty acids (NEFA), leptin, adiponectin, total ghrelin, high sensitive C-reactive protein (hs-CRP) and amino-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations.

Results: Arterial and venous concentrations of all factors analysed showed no statistical difference (all *p* values >0.1); leptin A: 39 ± 16 vs. V: 42 ± 18 ng/mL; total ghrelin A: 0.86 ± 0.27 vs. V: 0.76 ± 0.35 ng/mL; adiponectin A: 7.7 ± 3.3 vs. V: 7.7 ± 3.6 µg/mL; insulin A: 17.9 ± 9.7 vs. V: 18.6 ± 10.5 µU/mL; glucose A: 8.3 ± 2.1 vs. V: 7.9 ± 2.2 mM; NEFA A: 0.98 ± 0.93 vs. V: 0.89 ± 0.38 mM; hs-CRP A: 10.17 ± 7.68 vs. V: 10.27 ± 7.30 µg/mL and NT-proBNP A: 54.3 ± 47.9 vs. V: 54.7 ± 49.3 pg/mL.

Conclusion: These results suggest that radial artery and superior vena cava blood collection sites are comparable and may be used clinically with respect to fasting glucose, NEFA, leptin, adiponectin, total ghrelin, hs-CRP and NT-proBNP concentrations in a group of severely obese patients.

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The prevalence of obesity has increased over the last two decades and, alarmingly, a dramatic rise in the severe classes of obesity has been reported [1,2]. Severe obesity is associated with a cluster of comorbidities, affecting many systems of the body, including the cardiovascular system. Therefore, the severely obese patients are becoming a significant population in the field of cardiology. In the search to understand the pathophysiological mechanisms of obesity, there is a growing interest regarding neural, metabolic and endocrine peripheral signals that may regulate appetite and body weight. Molecules involved in this peripheral signaling are derived from either adipose tissue, including leptin and adiponectin, or from the gastrointestinal tract, such as ghrelin, and are released into the blood stream. Concentrations of these hormones may be influenced by insulin and glucose [3] as well as by non-esterified fatty acids (NEFA) [4], and may be modulated by inflammatory factors such as high sensitive C reactive protein (hs-CRP) [5]. The strong association between obesity and cardiovascular disease is of considerable interest [6]. Growing evidence suggests that leptin, adiponectin and ghrelin may be specifically implicated in the relationship between obesity and cardiovascular disease and that these signalling molecules may have an impact on amino-terminal pro-brain natriuretic peptide (NT-proBNP) levels [7]. Further studies are needed to explore their roles [8-12].

In the clinical setting, drawing blood samples from severely obese patients, in order to assess the circulating concentration of these molecules, may be difficult. Since many of these patients undergo invasive interventions, such as percutaneous transluminal coronary angioplasty (PTCA), cardiologists have access to an arterial line, facilitating blood sampling; however, most publications addressing leptin, adiponectin and ghrelin levels report results from blood obtained from venous sources [8,9,11]. The impact of the sampling site on the values of various laboratory analyses has been repeatedly debated [13-15]. Thus, the purpose of this study was to compare the circulating concentrations of leptin, adiponectin, total ghrelin, glucose, insulin, NEFA, NT-proBNP, and hs-CRP obtained in a clinical context where there is the potential for blood samples to be collected from arterial or venous sites.

Methods

Subjects

Randomly selected men and women, 18 years of age or older, with an indication for bariatric surgery (BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with associated comorbidities) were invited to participate in this study. Subjects who had previously under-

gone bariatric surgery or those bearing a pacemaker were excluded (as per manufacturer's safety indication, a patient with a pacemaker cannot undergo electrical bioimpedance assessment for adiposity measurement). The experimental protocol was approved by the ethics committee of the *Institut universitaire de cardiologie et de pneumologie de Québec* and all patients gave their written informed consent.

Anthropometric measurements

Height was measured using a stadiometer (SECA, 216 1814009, Brooklyn, NY, USA). Total body mass, body mass index (BMI), lean and fat masses were evaluated with an electrical bioimpedance balance (Tanita TBF-310, Tokyo, Japan) following a 12 hour fast.

Blood sampling

In preparation for bariatric surgery, the anaesthesiologist installed an arterial catheter in the radial artery and a central venous catheter in either the jugular or the subclavian vein, depending on the accessibility. A 12-15 cm long catheter was inserted into the superior vena cava, close to the heart's right atrium, for venous samples. Shortly after induction, but before beginning the surgical procedure, blood was simultaneously withdrawn from both line accesses (arterial and venous) in two syringes and immediately transferred into 6 mL tubes containing K₂EDTA. Tubes were rapidly placed on ice until centrifugation. Samples were centrifuged for 15 minutes at 3500 rpm, at 4°C within 15 minutes following collection. The separated plasma was collected and frozen in 2 mL aliquots at -80°C until analysis.

Plasma analysis

Concentrations of glucose and NEFA were measured using colorimetric enzymatic assays from Wako (Richmond, VA, USA). Insulin, adiponectin, leptin and total ghrelin concentrations were measured using radioimmunological assays according to manufacturer's protocol (Millipore, MA, USA). High sensitive C-reactive protein (hs-CRP) levels were measured using a commercially available ELISA according to manufacturer's protocol (Millipore, MA, USA). NT-proBNP concentrations were analyzed with a Modular system from Roche Diagnostics, using Roche reactants (Roche Diagnostics, IN, USA).

TABLE 1. Anthropometric data

N	15
Age (years)	40±10
Men/Women (N)	4/11
Weight (kg)	131.8±21.7
BMI (kg/m ²)	50.2±6.7
Fat percentage (%)	51.5±6.1
Fat mass (kg)	68.3±16.7
Fat free mass (kg)	63.4±9.6

Mean ± standard deviation

BMI: body mass index

Statistical Analysis

Data are presented as the arithmetic mean ± standard deviation. Comparisons between arterial and venous concentrations of designated molecules were analysed using the Student's paired t-test. A Wilcoxon Signed Rank test was performed when normality test failed. A p value <0.05 was considered statistically significant. Data were analysed using the statistical packages Sigma Stat 3.0 (Chicago, IL, USA).

Results*Anthropometric parameters*

Table 1 presents the anthropometric characteristics of the fifteen severely obese patients recruited in our study: 4 men and 11 women. Mean age was 40 ± 10 years. Weight of the subjects was 131.8 ± 21.7 kg with a BMI of 50.2 ± 6.7 kg/m². Average fat free mass was 63.4 ± 9.6 kg and fat mass was 68.3 ± 16.7 kg, representing 51.5 ± 6.1 % of the total body mass.

Arterial and venous blood concentrations

Table 2 presents the arterial (A) and venous (V) blood concentrations of leptin, total ghrelin, adiponectin, insulin, glucose, NEFA, hs-CRP and NT-proBNP. No statistically significant differences were observed between arterial and venous blood concentrations for any of these molecules (all p values >0.1). As an example, Figure 1 shows arterial and venous concentrations of glucose. While there were slight individual variations between arterial and venous samples, there was no statistical difference between collection sites (p=0.362).

Discussion

In a group of severely obese patients undergoing bariatric surgery, circulating concentrations of leptin, total ghrelin, adiponectin, insulin, glucose, NEFA, NT-proBNP, and hs-CRP

FIGURE 1. Arterial and venous concentrations of glucose

TABLE 2. Arterial and venous concentrations of hormones, substrates and inflammation marker

	Arterial	Venous	p value
N	15	15	
Leptin (ng/mL)	39 ± 16	42 ± 18	0.429
Total ghrelin (ng/mL)	0.86±0.27	0.76±0.35	0.409
Adiponectin (µg/mL)	7.7 ± 3.3	7.7 ± 3.6	0.954
Insulin (µU/mL)	17.9±9.7	18.6±10.5	0.425
Glucose (mM)	8.3±2.1	7.9±2.2	0.362
NEFA (mM)	0.98±0.93	0.89±0.38	0.135
hs-CRP (µg/mL)	10.17±7.68	10.27±7.30	0.679
NT-proBNP (pg/mL)	54.33± 47.9	54.67± 49.3	0.966

Mean ± standard deviation;

Student's paired t-test

NEFA: non-esterified fatty acids

hs-CRP: high sensitivity C-reactive protein

NT-proBNP: amino-terminal pro-brain natriuretic peptide

concentrations in blood taken from the radial artery compared to blood taken from the superior vena cava were comparable and clinically acceptable. The impact of collection site on blood concentrations of various molecules have been questioned in previous studies. Neptun *et al.* [16] reported that, in rats, the sampling site and collection method may be a major source of variation in clinical chemistry measurements, including those of glucose, cholesterol, triglycerides, ions and others. In canines, complete blood count obtained from venous and arterial blood samples were not comparable, yet values for haemostatic parameters with the exception of fibrinogen and thrombin time were similar [17]. In humans, it was also reported that in venous blood, there was a significant increase in erythrocyte count (2.7%) and haematocrit (3.1%) compared to arterial blood [18]. Furthermore, arterial and venous lactate levels showed discrepancies [13,19]. Thus, depending on a given parameter, the impact of sampling site may be important.

It is important to note that both sampling sites in this study represent general circulating levels and not tissue-specific levels. Most studies reporting arteriovenous differences in the levels of the molecules in this study were conducted for the purpose of measuring production or excretion by a specific organ. This is the case for many studies regarding glucose [20-29], insulin [30-32], NEFA [22], leptin [33-35] and NT-ProBNP levels [36]. Consequently, the results of these studies may not be comparable to our results because of different experimental designs.

To our knowledge, Evron *et al.* [13] is the only group who used a study design similar to the one reported here. In a cohort of 100 patients undergoing orthopaedic or colon surgery, blood was simultaneously sampled from three sites: 1) peripheral vein, 2) central vein and 3) radial artery after induction of anaesthesia. Concentrations of hemoglobin, electrolytes, pH, blood gases, lactate and glucose were measured and no statistically significant differences were found, with the exception of lactate. While studies on arteriovenous differences used different protocols, a few studies used peripheral sites bringing some information relevant to our study. Fontana *et al.* [30] reported arteriovenous concentration differences for insulin in the portal vein vs. peripheral artery blood in 25 severely obese subjects undergoing gastric bypass surgery. Fasting plasma insulin concentration in the portal vein was more than twice that in the peripheral artery (34.4 ± 21 and 15.2 ± 8 $\mu\text{U/mL}$ respectively). Similar observations were also reported by Horwitz *et al.* [37], who observed higher insulin concentrations in the portal vein compared with an antecubital vein in six non-diabetic subjects. It is important to note that insulin is extracted by the liver. Our results regarding insulin concentrations (A: 17.93 ± 9.70 and

V: 18.57 ± 10.46 $\mu\text{U/mL}$) are comparable to those of Fontana *et al.* [30] measured in a peripheral artery (15.2 ± 8 $\mu\text{U/mL}$) in a similar population. Venous and arterial insulin concentrations have never been compared. The results reported here complement published data since central venous and peripheral arterial insulin levels were within the same range.

In this study, venous blood was collected from the superior vena cava, which primarily drains the brain and the brachial adipose tissue and muscle. Esler *et al.* [35] reported that there was no detectable net flux of leptin through the forearm, results that have also been supported by Henriksen *et al.* [33] who found that in normal subjects there is a significant spillover of leptin into the iliac vein but not in the cubital vein. Esler *et al.* [35] also reported that leptin levels were higher in the internal jugular vein than the arterial value, suggesting a production of leptin by the brain in 15 lean to obese men (BMI range 19.6 - 38.5 kg/m^2). In contrast, no significant differences in leptin levels were observed in this severely obese population. The lack of details on the specific arterial sampling site and on the study population in the publication of Esler *et al.* [35] renders it difficult to reconcile their results with ours. Arteriovenous differences in ghrelin concentrations were also reported by Moller *et al.* [38] in 22 lean subjects (BMI of 23.6 ± 0.6 kg/m^2). Femoral artery concentration was 0.960 ± 0.082 ng/mL , while hepatic vein level was 1.102 ± 0.090 ng/mL , a 15% difference suggesting that the splanchnic bed is a major source of ghrelin [38]. Although the population in this study was severely obese, the concentrations of total ghrelin from the venous (0.76 ± 0.35 ng/mL) and the arterial (0.86 ± 0.27 ng/mL) sites were comparable to the artery site results reported by Moller *et al.* [38]. In support of our data, Goodyear *et al.* [39] recently reported no significant differences between central venous, arterial and peripheral venous sampling sites for either acylated ghrelin, des-acyl ghrelin or total ghrelin concentrations in humans. In their study of severely obese subjects, Fontana *et al.* [30] reported no significant difference in circulating levels of adiponectin between blood sampled from the portal vein and the radial artery. Consistent with these data, no statistically significant difference was seen here between venous and arterial adiponectin levels. Although Palsgaard-Van Lue *et al.* [17] found no statistical difference in hs-CRP values in arterial and venous blood samples in canines, no previous data have yet been reported in humans.

We acknowledge that this study included a small number of specifically selected severely obese patients and therefore our results may not be applicable to other population. Although it is clear that there is inevitable inter-individual variability in the parameters measured, it is of importance to emphasize that

each patient of our study was his/her own control, thereby reducing intra-individual variability.

Conclusion

The collection site, namely radial artery and superior vena cava, does not significantly influence clinical concentrations of leptin, total ghrelin, adiponectin, insulin, glucose, NEFA, hs-CRP and NT-proBNP in a group of severely obese patients. Our results suggest that for severely obese patients, for whom it is difficult to obtain antecubital venous blood samples or for whom arterial access would be more convenient (such as during PTCA or surgery), blood samples may be withdrawn from either radial artery or superior vena cava for the measurement of leptin, total ghrelin, adiponectin, insulin, glucose, NEFA, hs-CRP and NT-proBNP levels.

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List of Abbreviations

A	arterial blood
V	venous blood
NEFA	non-esterified fatty acids
hs-CRP	high sensitive C-reactive protein
NT-proBNP	amino-terminal pro-brain natriuretic peptide
PTCA	percutaneous transluminal coronary angioplasty
BMI	Body mass index

References

- Katzmarzyk PT, Mason C. Prevalence of class I, II and III obesity in Canada. *CMAJ*. 2006;174:156-157.
- Sturm R. Increases in morbid obesity in the USA: 2000-2005. *Public Health*. 2007;121:492-496.
- Boden G, Chen X, Mozzoli M et al. Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab*. 1996;81:3419-3423.
- Dubuc GR, Phinney SD, Stern JS et al. Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. *Metabolism*. 1998;47:429-434.
- De Rosa S, Cirillo P, Pacileo M et al. Leptin stimulated C-reactive protein production by human coronary artery endothelial cells. *J Vasc Res*. 2009;46:609-617.
- Poirier P, Giles TD, Bray GA et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation*. 2006;113:898-918.
- Pervanidou P, Margeli A, Akalestos A et al. Associations between circulating N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) and adiponectin concentrations depend on obesity level in female adolescents: gender dimorphic findings. *Horm Metab Res*. 2009;41:829-833.
- Perego L, Pizzocri P, Corradi D et al. Circulating leptin correlates with left ventricular mass in morbid (grade III) obesity before and after weight loss induced by bariatric surgery: a potential role for leptin in mediating human left ventricular hypertrophy. *J Clin Endocrinol Metab*. 2005;90:4087-4093.
- Quilliot D, Bohme P, Zannad F et al. Sympathetic-leptin relationship in obesity: effect of weight loss. *Metabolism*. 2008;57:555-562.
- Katergari SA, Milousis A, Pagonopoulou O et al. Ghrelin in pathological conditions. *Endocr J*. 2008;55:439-453.
- Tritos NA, Kissinger KV, Manning WJ et al. Association between ghrelin and cardiovascular indexes in healthy obese and lean men. *Clin Endocrinol (Oxf)*. 2004;60:60-66.
- Shibata R, Ouchi N, Murohara T. Adiponectin and cardiovascular disease. *Circ J*. 2009;73:608-614.
- Evron S, Tress V, Ezri T et al. The importance of blood sampling site for determination of hemoglobin and biochemistry values in major abdominal and orthopedic surgery. *J Clin Anesth*. 2007;19:92-96.
- Keidar S, Aviram M, Grenadier E et al. Arterial blood derived low density lipoprotein increases platelet aggregation and macrophage cholesterol content in comparison to lipoprotein derived from venous blood. *Artery*. 1989;16:62-71.
- Russell AE, Aylward PE, Smith SA et al. Plasma neuropeptide Y and atrial natriuretic peptide concentrations in man. *Clin Exp Pharmacol Physiol*. 1988;15:955-958.
- Neptun DA, Smith CN, Irons RD. Effect of sampling site and collection method on variations in baseline clinical pathology parameters in Fischer-344 rats. 1. Clinical chemistry. *Fundam Appl Toxicol*. 1985;5:1180-1185.
- Palsgaard-Van Lue A, Jensen AL, Strom H et al. Comparative analysis of haematological, haemostatic, and inflammatory pa-

- rameters in canine venous and arterial blood samples. *Vet J*. 2007;173:664-668.
18. Yang ZW, Yang SH, Chen L et al. Comparison of blood counts in venous, fingertip and arterial blood and their measurement variation. *Clin Lab Haematol*. 2001;23:155-159.
 19. Gallagher EJ, Rodriguez K, Touger M. Agreement between peripheral venous and arterial lactate levels. *Ann Emerg Med*. 1997;29:479-483.
 20. Baron AD, Brechtel G, Wallace P et al. Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am J Physiol*. 1988;255:E769-E774.
 21. Baron AD, Laakso M, Brechtel G et al. Reduced postprandial skeletal muscle blood flow contributes to glucose intolerance in human obesity. *J Clin Endocrinol Metab*. 1990;70:1525-1533.
 22. Coppack SW, Frayn KN, Humphreys SM et al. Arteriovenous differences across human adipose and forearm tissues after overnight fast. *Metabolism*. 1990;39:384-390.
 23. Myers JD. Net splanchnic glucose production in normal man and in various disease states. *J Clin Invest*. 1950;29:1421-1429.
 24. Ekberg K, Landau BR, Wajngot A et al. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. *Diabetes*. 1999;48:292-298.
 25. Cano N. Bench-to-bedside review: glucose production from the kidney. *Crit Care*. 2002;6:317-321.
 26. Coppack SW, Frayn KN, Humphreys SM et al. Effects of insulin on human adipose tissue metabolism in vivo. *Clin Sci (Lond)*. 1989;77:663-670.
 27. Baron AD, Tarshoby M, Hook G et al. Interaction between insulin sensitivity and muscle perfusion on glucose uptake in human skeletal muscle: evidence for capillary recruitment. *Diabetes*. 2000;49:768-774.
 28. DeFronzo RA, Sherwin RS, Felig P. Synergistic interactions of counterregulatory hormones: a mechanism for stress hyperglycemia. *Acta Chir Scand Suppl*. 1980;498:33-42.
 29. Coppack SW, Fisher RM, Humphreys SM et al. Carbohydrate metabolism in insulin resistance: glucose uptake and lactate production by adipose and forearm tissues in vivo before and after a mixed meal. *Clin Sci (Lond)*. 1996;90:409-415.
 30. Fontana L, Eagon JC, Trujillo ME et al. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes*. 2007;56:1010-1013.
 31. Chamberlain MJ, Stimmler L. The renal handling of insulin. *J Clin Invest*. 1967;46:911-919.
 32. Samols E, Ryder JA. Studies on tissue uptake of insulin in man using a differential immunoassay for endogenous and exogenous insulin. *J Clin Invest*. 1961;40:2092-2102.
 33. Henriksen JH, Holst JJ, Moller S et al. Elevated circulating leptin levels in arterial hypertension: relationship to arteriovenous overflow and extraction of leptin. *Clin Sci (Lond)*. 2000;99:527-534.
 34. Klein S, Coppack SW, Mohamed-Ali V et al. Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes*. 1996;45:984-987.
 35. Esler M, Vaz M, Collier G et al. Leptin in human plasma is derived in part from the brain, and cleared by the kidneys. *Lancet*. 1998;351:879.
 36. Palmer SC, Yandle TG, Nicholls MG et al. Regional clearance of amino-terminal pro-brain natriuretic peptide from human plasma. *Eur J Heart Fail*. 2009;11:832-839.
 37. Horwitz DL, Starr JI, Mako ME et al. Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest*. 1975;55:1278-1283.
 38. Moller N, Nygren J, Hansen TK et al. Splanchnic release of ghrelin in humans. *J Clin Endocrinol Metab*. 2003;88:850-852.
 39. Goodyear S, Arasaradnam RP, Quraishi N et al. Acylated and des acyl ghrelin in human portal and systemic circulations. *Mol Biol Rep*. 2010;37:3697-3701.